

THE EFFECTS OF LIGHT ON TEMPERATURE SELECTION IN
SPECKLED TROUT *SALVELINUS FONTINALIS*
(MITCHILL)¹

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It will be generally accepted that the locomotory behavior of any animal in its ordinary life is a reaction or response resulting from the host of stimuli both internal and external which are continually acting upon it. The physiological analysis of such resultants is so obviously complex that here, as in most other fields, investigators have felt it desirable to commence investigation in simplified situations where the number of independent variables operating simultaneously is kept as few as possible. As a consequence much has been learned about the reactions of a great variety of animals to light, when temperature, gravity, humidity, nutritional state, etc., are kept constant; of the reaction to temperature if light and the other possible variables are kept constant, and so on. The relatively enormous literature which has resulted has been admirably summarized by Fraenkel and Gunn (1940).

It is evident, however, that if a complete understanding is to be obtained of the sensori-motor mechanisms and the nervous system which integrates them, experiments will be required in which the reactions to the simultaneous application of several stimuli are studied. Initial steps in this direction have already been made in a number of instances. Even Mendelsohn (1902) in his early work with protozoa showed that the responses of the organisms to two simultaneously acting stimuli were varied and depended upon the experimental arrangements. With the proper conditions the stimuli reinforced one another. Under other conditions the effect of one or the other stimulus prevailed—to a degree which was related to the relative strengths of the two stimulating conditions.

Of the stimuli which can be combined, light and temperature are among the most easily controlled from an experimental standpoint and are possibly among the most important factors affecting organisms from moment to moment. It is well known that when organisms are free to move about in a gradient of temperature they tend to be confined to some particular relatively small range of temperature which they are then said to have "selected." (See Fraenkel and Gunn, 1940; Sullivan and Fisher, 1953; Stinson and Fisher, 1953, for references.) It is equally well established that given both a lighted and a darkened area many organisms select one or the other (Fraenkel and Gunn, 1940; for fish specifically see Schiche, 1921; Young, 1935; Breder and Rasquin, 1950; Collins, 1952 and Lowe, 1952). There are instances in which some particular intensity was selected in a gradient of light intensity (Weber, 1929; Henschel, 1929; Totze, 1933; Ulliyott, 1936 and Herrström, 1949).

¹ This investigation was assisted by a grant from the National Research Council of Canada.

The interrelations of the effects of light and temperature are clearly of interest. Some have already been described. Thus Herter (1923b) reported that house crickets selected a higher temperature in diffuse daylight than they did when exposed to lower levels of illumination, while red ants (Herter, 1923b, 1924) and flower beetles (Bodenheimer and Schenkin, 1928) selected a lower temperature in daylight than when less intensely illuminated. Actually there are apparently some instances in which no interrelation exists. Thus Herter (1923a, 1924) found that the temperatures selected by fireflies and field crickets, respectively, were independent of the incident light intensity.

The distribution of organisms in such a non-uniform environment as a temperature gradient is characterized not only by its mode, which is taken as the condition selected, but also by its spread. The latter indicates the sharpness or precision of the selection and has been measured by the standard deviation of the distribution. Herter (1923b, 1924) showed in this way that the precision of temperature selection by adults of *Acheta domestica* and *Lyogryllus campestris*, respectively, was greater (*i.e.*, the standard deviation was smaller) in light than in the dark. Although Bodenheimer and Schenkin (1928) do not discuss this point, the standard deviations of the distributions they observed for the flour beetle and for *Scanthius aegyptius* were not the same in darkness and in light. In these cases temperature selection appeared more precise in the dark than in the light.

The effect of the ambient light intensity on temperature selection by fish does not appear to have been examined, although the reverse experiment, the effect of different constant temperatures on light selection has been reported (*cf.* Andrews, 1946 and the preliminary work by Collins, 1952). Moreover, although the behavior of fish in response to simultaneously operating factors has been examined in certain connections, the distribution of fish in an environment in which there were non-uniformities of light as well as of temperature does not appear to have been studied. We have therefore determined the selected temperature of trout and the precision of selection at two light intensities and, because it was a necessary prerequisite, the precision of selection in successive experiments with the same fish; data were also obtained which establish the nature of the distribution to be expected in an environment in which non-uniformities of light and temperature are superimposed.

MATERIALS AND METHODS

The experimental animals were young speckled trout, *Salvelinus fontinalis* Mitchill, two to three inches long. They were obtained from a private hatchery² and held in running Toronto tap water.

For all the experiments to be discussed, a five-foot long copper-bottomed trough with glass sides was used. This apparatus, the method of establishing and maintaining a temperature gradient in it and the procedure followed to determine the temperature selected by the experimental organisms have been described previously (Sullivan and Fisher, 1953).

For the experiments to be reported here the trough was provided with an arrangement of lights and opaque light shields ("covers") by means of which the desired lighting conditions were set up. A diagram showing the trough and the

² It is a pleasure to acknowledge the cooperation of Mr. Herbert Pearen of Hornings Mills, Ontario, in this connection.

light shields in cross section will be found in Figure 1. The trough in which the fish were placed is designated as (a). Shield (b) was closely applied to the back of the trough (a), and effectively prevented any entrance of light from that direction. Shield (c) similarly covered the front of the trough except for a longitudinal slit along the bottom which permitted observation of the fish in the trough. Shield (d) was made of several overlapping sections so that by removing appropriate sections and adjusting the remaining ones any desired region of the trough could be illuminated. To make the boundary between the lighted regions and the dark

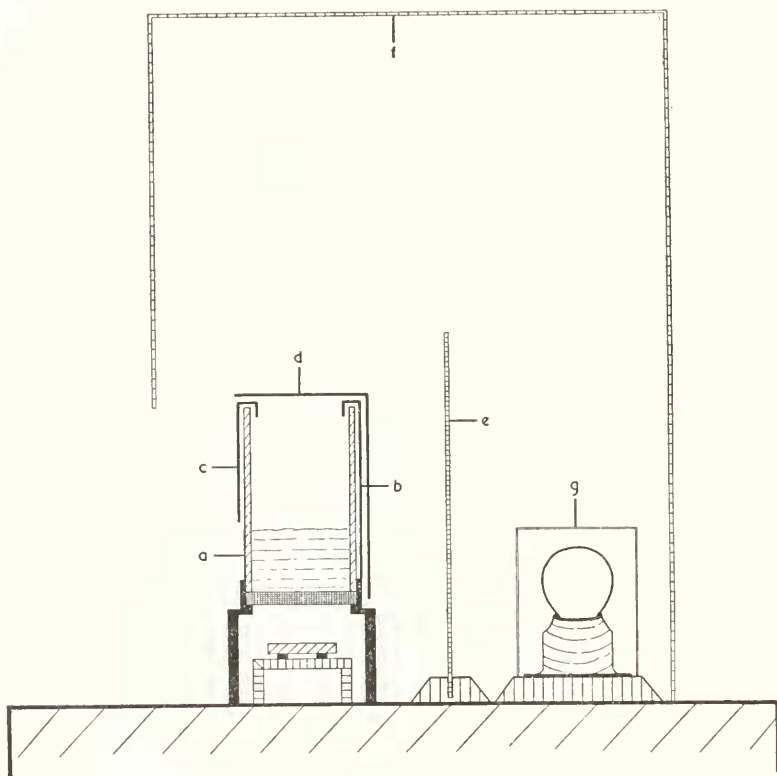


FIGURE 1. Diagram of trough and light shields; cross-section.

regions as sharp as possible, vertical shields were hung from the edges of cover (d) to within $\frac{1}{2}$ inch of the surface of the water in the trough.

Light was provided by a row of six electric bulbs evenly spaced along the back of the apparatus. The illuminated surfaces of shields (e) and (f) were left their natural color (light buff) so as to provide diffuse reflection of light into the uncovered regions of the trough. All other surfaces were painted flat black.

Two types of experiments were done. For the first type, in which the whole trough was evenly illuminated, all the sections of shield (d) were removed. Under these circumstances "bright" light was provided by using 25-watt bulbs in the six sockets; "dim" light was obtained by using $7\frac{1}{2}$ -watt bulbs and covering the whole row of bulbs with thin white cardboard ((g) in Fig. 1).

For the second type of experiment, in which only part of the trough was illuminated, the required region was uncovered by adjusting shield (d). Six 25-watt bulbs were used. The intensity of light in the uncovered region was reduced as required by placing one or more layers of white paper over the opening in shield (d).

RESULTS

The effect of the incident light intensity on the selection of temperature may be determined either by studying a given group of fish at each of the light intensities to be used, or by using several groups of fish each of which is studied at only one intensity. The first of these procedures involves the subjection of a group of fish to two or more light intensities in succession. In it there is clearly a possibility that the behavior in the second and any subsequent exposures in the gradient may be affected by the previous experience in the gradient. A serial effect of this kind is of interest, of course, not only because it might affect the interpretation of the data obtained by procedure one, but also for its own sake, since the existence of any such serial effect would be indicative of a kind of learning by the organisms. The first experiments to be discussed here provide data about the serial effect in gradient experiments.

Effect of successive exposures to the temperature gradient

Each of nine groups of fish was studied upon three or more occasions, these being separated by one week or more. The light intensity used ("bright," see Methods) was constant along the gradient and was the same throughout all of these experiments. Typically the histogram which described the distribution of the fish in each experiment was definitely peaked. There was a considerable variation in the number of observations in the modal group from day to day and from group to group of experimental animals. It was not possible, however, to detect any consistent change in the value of the temperature selected from trial to trial other than those that normally occur with changing seasons (Sullivan and Fisher, 1953).

It seems then that as far as the actual temperature selected was concerned there was no serial effect. The sharpness of the selection on the other hand, as indicated by the standard deviations of the various distributions, did appear to change with successive determinations. These standard deviations are listed in Table I and it is evident from the mean values that the distributions tended to be spread out more on the temperature axis in the initial trial than in subsequent ones. Moreover the tendency for the standard deviation to decrease in successive trials may be seen in each of the individual groups of fish with the exception of groups 4 and 6—and it is particularly apparent in the cases of groups 1, 2 and 3, which were each observed in four successive experiments. The mean value for the nine groups in trial 1 is significantly different, in the statistical sense, from the mean value in trial 2 ($t = 2.5$, $n = 8$, probability 4%). The mean value for the groups in trial 1 is likewise different from that in trial 3 ($t = 2.6$, $n = 8$, probability 4%).

In these experiments, then, the standard deviation of the distribution of a group of fish in the gradient decreased somewhat with successive tests up to the third or fourth such tests. It is not at all apparent how this could have been an artifact resulting from the experimental conditions and so it must be concluded that it was a property of the organisms themselves. Experience in the gradient *per se* or be-

TABLE I

Standard deviations of selection histograms obtained in successive trials of nine groups of fish in temperature gradients

Group	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
1	4.38	3.48	3.53	3.02	3.02
2	4.95	5.07	4.71	4.32	
3	4.56	4.08	3.96	3.60	
4	2.94	2.91	3.09		
5	3.44	3.36	2.84		
6	4.23	4.23	4.17		
7	4.62	3.84	4.38		
8	4.62	3.99	3.75		
9	4.68	3.24	3.02		
Mean	4.27	3.80	3.72	3.68	

cause of the development in the fish of a familiarity with the apparatus led to a sharper, *i.e.*, more precise, selection of temperature.

Effect of incident light intensity on temperature selection

The experiments made to determine the precision of temperature selection in bright and dim light involved four different groups of fish, each consisting of ten individuals. Each group was divided into two lots, designated (a) and (b), respectively. Lot (a) of each group was examined first in dim light and then, in a second experiment, in bright light, while lot (b) was examined first in bright

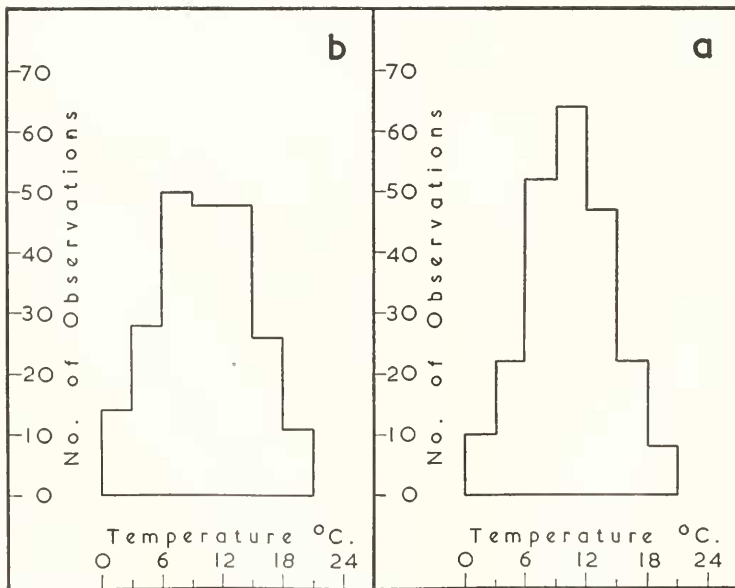


FIGURE 2. Distribution of trout in the temperature gradient. a) gradient brightly lighted; b) gradient dimly lighted.

light and then, in a second experiment, in dim light. These two determinations of the selected temperature were the only ones made on each of the two lots of groups one, two and three. A third determination was made, however, in the case of the two lots of group four, and for this determination each lot was exposed again to the initial illumination condition which it had experienced.

Histograms showing the average distribution of the organisms in dim and bright light respectively are shown in Figure 2a and b. It may be seen that a more peaked distribution occurred in the dim light (Fig. 2a). The standard deviation of the distribution in Figure 2a is 4.08 as compared with 4.62 in Figure 2b. Apparently the precision of selection was greater in the dim than in bright light. Such experiments as these which have just been discussed involved subjection to two different light intensities successively, and, as was noted above, repetition of temperature selection experiments tends to elicit sharper selection. As a consequence it could be expected that the apparent difference between the sharpness of

TABLE II

Group	Dark	Light	Time in gradient
1	(a) 4.11 3.87	(b) 5.46 4.98	In this series 1a and 1b were tested one day and five days later 1a was tested twice, first in light, then in dark
2	(a) 3.46 3.81	(b) 4.86 4.23	First Second
3	(a) 4.71 4.02	(b) 4.83 4.20	First Second
4	(a) 4.35 3.96 3.72	(b) 4.59 4.56 3.96	First Second Third

selection in dim and bright light would be greater when the first determination was made in bright light than when it was made in dim light. In the one case, the effect of repetition would reinforce the change due to the difference in light intensity, while in the other, it would tend to cancel it. It is of interest, therefore, to examine the standard deviations of the individual distributions which were combined to produce Figure 2. They are given in Table II. The values joined by arrows were determined on the same lot of fish, the sequence of the experiments in light and dark being indicated by the direction of the arrows.

If one examines the instances in which the determination was made first in bright and then in the dim illumination (one instance for each of groups 2 and 3 and two instances for group 4) it may be seen that without exception the standard deviations are considerably greater in bright than in dim light. When, however, the instances are taken in which a given group of fish was observed first in dim and then in bright light, the standard deviation in the second experiment is greater than that in the first in only one instance (Group 3a). In one case (4a, second and third trials) the standard deviations are the same while in the remaining three

cases the standard deviation of the first determination in dim light is actually considerably less than that of the second experiment in bright light.

As was to be expected then, the serial effect enhanced, if anything, the change in standard deviation seen when the organisms were examined first in bright light but it reduced or reversed the change due to lighting conditions when the experiments in dim light were done first.

There is then no question that a given lot of fish select more sharply in dim light than in bright light and it is evident that the serial effect operates even though the additional factor of light intensity is also involved.

These experiments in which the standard deviation of the temperature selection distribution were determined at the different light intensities also provide information about the temperature selected at the two light intensities. Nine comparisons of the temperature selected by the (a) lots at one light intensity with the temperature selected by the (b) lots at the other intensity may be made.

The pairs of values, in ° C., were as follows, the top member of each pair being the observations in bright light in each case:

13.2	13.2	11.5	8.1	12.2	10.3	10.5	7.5	7.8
13.4	13.4	10.9	8.1	10.5	10.6	10.5	7.9	9.0

The mean value at both intensities was 10.5° C. It may be concluded that the temperature selected by brook trout is not dependent on the ambient light intensity within the limits of intensity used here. The selected temperatures listed for both bright and dim light do show a trend toward lower values, because the determinations were made in the autumn when a seasonal shift in the temperature selected by trout regularly occurs (Sullivan and Fisher, 1953).

Interference between selection of light and temperature

For this part of the study two experiments, *i.e.*, determinations of the distribution of five organisms in the experimental temperature gradient, were done each day. In the first or control experiment a group of five fish was observed for two hours (five fish, each located forty-eight times, making a total of two hundred and forty observations) in the temperature gradient with the gradient trough completely covered. In this experiment the selected region was established. In the second experiment a comparable group of organisms from the same laboratory stock was used, and the region selected in the control experiment was exposed to the desired illumination. The highest intensity employed will be arbitrarily designated 100% (this corresponds to an incident illumination density of approximately 15 foot candles); other intensities will be specified relative to this. It may be recorded here that as a consequence of the particular light arrangements used in these experiments (described under Methods) the intensity of light in the darkened regions of the trough was found to be practically independent of the intensity in the lighted region. The actual intensity of the darkened parts, as well as the intensity of the whole trough when it was uniformly lighted with "dim" light was approximately 1% on the arbitrary scale.

Typical examples of the distributions found with the selected region (always taken to be one third of the length of the gradient trough) illuminated at the

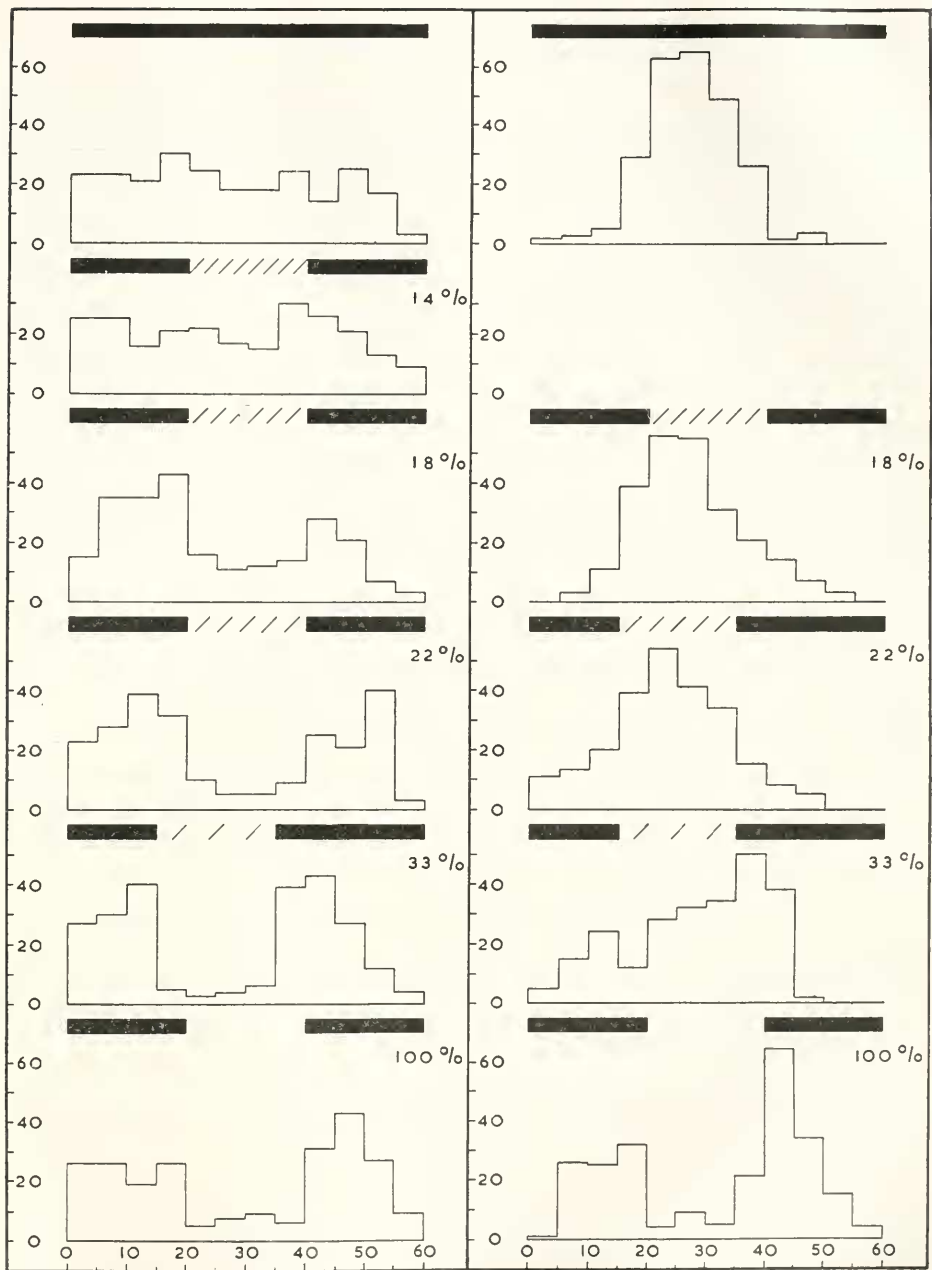


FIGURE 4.

FIGURE 3.

FIGURE 3. Distribution of trout in the temperature gradient. The horizontal axis represents inches along the trough and the vertical axis represents frequency of observations of fish. Light conditions in each experiment are indicated by the black bars. Illumination of a particular region of the trough is indicated by interruption of the black bar over that region. The relative intensity of illumination of the exposed region in each experiment is given by the figure at the upper right of each distribution.

FIGURE 4. Distribution of trout in the trough at constant temperature. For further explanation see the legend of Figure 3.

intensities indicated at the right of each histogram, respectively, are given in Figure 3. The distribution at the top of the figure was found when the trough was uniformly illuminated. The black bar indicates that the light was dim, *i.e.*, the whole trough was covered. Normal temperature selection took place. The distribution shown at the bottom of the figure resulted when the selected region was illuminated with the highest intensity. With this condition, as noted above, the density of illumination in the lighted region was approximately $100 \times$ that of the darkened region. It is apparent that this difference in intensity along the trough produced a marked effect. The region now illuminated rarely contained organisms although the latter were observed in it more frequently than elsewhere when the only non-uniformity was temperature. Those animals which did occasionally move into the lighted region characteristically swam rapidly through it to the shade on the other side. Most of the organisms were observed to stay just under the edges of the covers on either side of the lighted area, moving about quietly and thus giving rise to the distribution shown in the figure. It is evident that the reaction of the organisms to the non-uniformity of the illumination conditions was stronger than was the reaction to the non-uniformity of temperature.

With the selected region illuminated at an intensity of 33%, normal temperature selection did not occur although Figure 3 shows that the effect of the light was not as great as at an intensity of 100%. At intensities of 22% and 18%, it appeared that the light was not effective in keeping the organisms out of the selected region. Essentially normal temperature selection was found at these and lower levels of illumination.

These experiments did not show whether the fish were simply incapable of perceiving light at the lower intensities, or whether the response to light at these intensities was actually "overruled," as it were, in the central nervous system of the organisms by the reaction to temperature. To obtain information on this point some experiments were made with the trough at a uniform temperature of 11°C. , the middle third of the trough being illuminated at different intensities in exactly the same way as was done when a temperature gradient was used. Data typical of those obtained in such experiments are given in Figure 4. In each case the light intensity was the same as that used in the gradient experiment which appears beside it in Figure 3. It may be seen in Figure 4 that all intensities down to and including 18% of the maximum, effectively kept the trout out of the illuminated area. At an intensity of 14% they were relatively indifferent to the light and the distribution at this intensity was not significantly different from that found with the trough uniformly darkened and at a constant temperature.

It will be noted that although the organisms stayed out of intensities 18% and 22% when the trough was at a uniform temperature, they occupied areas illuminated at these intensities in the temperature gradient when the selected temperature was arranged to occur in them. It is thus evident that the failure of light at the 18 and 22% levels to modify the distribution in the temperature gradient was not the consequence of an inability to perceive or appreciate such intensities. Instead it must be concluded that there was competition between the responses to light and to temperature in the gradient. The intensity of light which the fish would enter in the presence of a temperature gradient was considerably greater than the intensity which they would enter if no gradient were present. At high light intensities the negative response to light predominated and the trout were kept out

of the selected region when it was lighted. As the intensity of light was reduced the relative effectiveness of the light and temperature stimuli on behavior changed, and at an illumination of approximately 20%, the response to temperature had come to predominate so that the animals selected temperature as though no non-uniformity of illumination existed.

The reactions to non-uniformities of light described above were interpreted as "selections" of dim light in preference to bright light. Theoretically, at least, this could have been incorrect. Instead, it might have been that in their movements the organisms tended to preserve the existing condition, dim or bright, rather than move across a demarcation line, from dim to bright, or vice versa. In other words, the fish may not actually have selected a dim light rather than a bright one. They may simply have "hesitated," as Breder (1951-52) expressed it, to move from the one condition, dim, to the other, bright. Should this be the case then it would follow that the selection of a particular region in a temperature gradient would be *less* sharp with the region in question shaded than it would be with the gradient uniformly illuminated. This, however, was not the case. When the region selected in the temperature gradient was shaded with the remainder of the trough highly illuminated, the fish selected very sharply—all remained under the cover. It must be concluded that these reactions to light are correctly interpreted as active selection of some intensities in preference to others; in general, selection of dim light in preference to bright light.

The fish used throughout the investigation reported above were exposed to the normal daily fluctuations of light intensity. The effect of eliminating these fluctuations for a period prior to such experiments as are described here was examined in two instances. For one of these a group of fish was placed in a white tank and exposed continuously for three weeks to the light of a 60-watt lamp placed about two feet above the water. For the second a comparable group of fish was kept in continuous darkness for three weeks. Each group was then tested in a gradient with the selected region illuminated and the rest of the trough darkened as usual. Both the light and the dark adapted fish were kept out of the selected region by 100% light and there was no detectable difference in the behavior or distribution of the two groups.

It would be expected that the effect of light in these experiments was mediated by the eyes. That this is in fact the case was demonstrated by experiments with blinded fish. These were found to select temperature perfectly normally even with the selected region exposed to the maximum intensity, the rest of the trough being in "darkness."

SUMMARY

1. The distribution of groups of five speckled trout in a longitudinal gradient of temperature was studied under a variety of conditions of illumination.

2. The modes of the distributions, *i.e.*, the selected temperatures, did not change with successive determinations separated by a week or more. However the selection of temperature became more precise as the fish acquired experience in the apparatus.

3. Selection of temperature was apparently more precise at low light intensities than at high intensities although the actual temperature selected did not vary with the light intensity.

4. Some experiments were done in which only part of the experimental environment was illuminated. When temperature was constant trout would not remain in the lighted part unless the intensity of illumination was very low. When, in a gradient of temperature, the illuminated part was made to coincide with the region normally selected by virtue of the temperature, it was found that at high light intensities the organisms did not appear in the illuminated region. Clearly a response to light prevailed. At intermediate and low light intensities, on the other hand, it was the response to temperature which prevailed and in spite of the illumination the organisms selected temperature in a normal way.

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